extent of the disease and different parameters from the patient. Typically, 0.001 to 50 mg/kg/day will be administered to the mammal.

The methods and assays of the present invention have also been validated with Annexin. This protein is significantly different from P-glycoprotein in both structure and function. Consequently, together with the knowledge of protein chemistry and molecular biology, these validations support the utility of the instant assays and methods for all proteins (from viruses, living cells, animals, plants, etc.)

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1 shows the principle of protein-protein interaction. The plus signs (+) indicate the regions of high affinity binding. The minus signs (--) indicate the regions of high-repulsive forces. As indicated in the text, interactions between two proteins are made up of discontinuous regions of high affinity binding and high-repulsive forces that are almost in equilibrium with high affinity binding being more favoured while proteins are together.

Figure 2 is a schematic representation of a method of identification of high affinity binding sequences according to one embodiment of the present invention. ~~A,~~ 2A the different shapes represent different proteins in a total cell lysate. The signs are like for Figure 1. ~~B,~~ 2B small overlapping peptides that cover the entire sequence (or a segment) of protein. A will be synthesized directly on derivatized wells of 96-well polypropylene plates. Following peptide synthesis, metabolically radiolabeled total cell lysate is added to each well containing the various peptides and incubated in an incubator buffer. ~~C,~~ 2C the dark filled circles represent the radiolabeled proteins from total cell lysate isolated from metabolically radiolabeled cells added to all the wells of the 96-well plates to identify high affinity binding sequences on Protein A. ~~D,~~ 2D after an extensive washing, the high affinity binding sequences (overlapping peptides from Protein A) are in those wells that bind radiolabeled proteins (in dark). Four high affinity binding sequences between Protein A and another protein(s) are identified in rows 1, 3, 6 and 8. The

wells that contain the high affinity binding sequences are identified by radiolabeled counting and SDS-PAGE.

Figure 3 is a schematic representation of a method of identification of high affinity binding sequences according to another embodiment of the present invention. 3A ~~A~~ shows a schematic representation of the interaction between Protein A and Protein B. 3B ~~B~~, small overlapping peptides that cover the entire sequence (or a segment) of Protein A will be synthesized directly on derivatized wells of 96-well polypropylene plates. Following peptide synthesis, a radiolabelled Protein B (synthesized from *in vitro* transcription-translation reaction mix) are added to each well containing the various peptides and incubated in an incubation buffer. 3C ~~C~~, the dark filled circles represent the radiolabeled Protein B that has been added to all the wells of the 96-well plates to identify high affinity binding sequences on Protein A. 3D ~~D~~, after a washing procedure, the high affinity binding sequences are in those wells in which Protein B (radiolabeled protein in dark) is still bound to the peptides from Protein A. ~~[E, four high affinity binding sequences between Protein A and Protein B are identified in rows 1, 3, 6 and 8. The wells that contain the high affinity binding sequences are identified by radiolabeled counting and SDS-PAGE.]~~

Figure 4 is a schematic representation of a method of selection of drugs that specifically inhibit the binding of protein A to B according to one embodiment of the present invention. 4A ~~A~~ shows a schematic representation of the interaction between Protein A and Protein B. 4B ~~B~~, peptides that encode high affinity binding sequences are used as LEAD sequences for the selection of specific drugs that inhibit the association between Protein A and Protein B and ultimately the function of the complex. To target the high affinity binding sequences that were identified in Figures 2 or 3, peptides encoding one of the high affinity binding sequences are synthesized in every well of the 96-well plate. Grey circles represent one of four high affinity binding sequences identified in Figures 2 and 3. 4C ~~C~~, following the addition of a compound to be tested to each well of the 96-well plate, a radiolabeled Protein B are added to each of the wells. Of course, combinatorial libraries can be screened to identify drugs that bind specifically to the high affinity binding sequences of Protein A. As previously stated, radiolabeled Protein B from transcription-translation reaction mix are represented. Plates are washed and drugs that

specifically bind to high affinity sequences of Protein A are found in those wells that do not contain radiolabeled Protein B. ~~D~~ wells containing drugs/compounds that bind specifically to one of the high affinity binding sequence in Protein A and therefore prevent the binding of Protein B are identified by the absence of a dark circle (*i.e.,* wells 28, 70 and 75). Selected drugs/compounds represent invaluable LEAD compounds that can be used in biological assays to confirm their mechanism of action. Validated drugs can proceed toward *in vivo* studies.

Figure 5 shows a P-glycoprotein predicted secondary structure and amino acid of the linker domain. A schematic representation of P-gp predicted secondary structure. The twelve filled squares represent the twelve putative transmembrane domains. The two ATP binding domains are represented by two circles in the N- and C-terminal halves of P-gp. The inset represents the linker domain. The amino acid sequence of the linker domains of Human P-gp 1 (HP-gp1) and HP-gp3 is indicated as a single-letter amino acid code. The numbers in brackets at the beginning and end of each amino acid sequence of HP-gp1 and HP-gp3 shows the length of the linker domains (1 - 90 and 1- 88 for HP-gp1 and HP-gp3, respectively). The numbered lines underneath the amino acid sequence show the sequences of the overlapping hexapeptides, which differ by one amino acid. For HP-gp3, the last hexapeptide is number 88.

Figure 6 A-C shows the protein binding to overlapping hexapeptides encoding HP-gp1 linker domain. Overlapping hexapeptides that encode the linker domain of HP-gp1 were synthesized on polypropylene rods and used to identify proteins that bind to these peptides. A total of 90 plus two control hexapeptides for HP-gp1 were incubated with total cell lysate from [^{35}S] methionine metabolically labeled cells (see methods). All bound proteins were eluted from the peptide-fixed rods and resolved on 10% SDS-PAGE. Lanes 1 to 92 show the [^{35}S] methionine bound proteins from HP-gp1. The migration of the molecular weight markers is shown to the left of gels.

Figure 7 shows the effects of different detergents or high salt on the binding of proteins to HP-gp1 hexapeptides. Metabolically radiolabeled proteins bound to hexapeptides (hexapeptides 50 to 53) from HP-gp1 linker domain were eluted in the presence of increasing concentrations of anionic detergent (0.12% - 0.5% SDS), zwitterionic detergent (20 mM - 80 mM CHAPS) or salt (0.3 M - 1.2 M KCl). The y-axis represents the amount of radioactivity eluted from a pool of three hexapeptides (50 to 53).